

CLAIMS

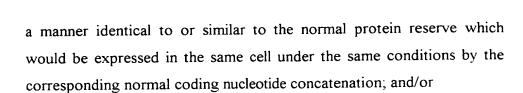
- 1. An oligonucleotide comprising at least one concatenation coding for a polypeptide with formula (P-K)_n, where:
 - n is a whole number of 2 or more;
 - P represents a proline amino acid residue,
 - K represent a lysine amino acid residue;
 - the symbol "4" represents a bond between the two amino acid residues, in particular a peptide type bond, the n (P-K) units also being bonded together by such bonds, for example peptide type bonds.
- An oligonucleotide according to claim 1, comprising a concatenation coding for a polypeptide with formula (P-K)_n where n is a whole number of 3 or more, and preferably n is equal to 4, 5, 6, 7, 8, 9, 10 or 15.
 - An oligonucleotide according to claim 1 or claim 2, comprising a concatenation coding for a polypeptide with formula (P-K)_n, in which the sequence of n (P-K) units is interrupted by one or more amino acid residues other than P or K residues.
 - An oligonucleotide according to any one of claims 1 to 3, characterized in that the concatenation coding for the polypeptide comprising the n (P-K) units is completed at its 5' end and/or at its 3' end by one or more codons coding, for example, for at least one lysine residue at the N-terminal extremity of the formed polypeptide.
 - 5. An oligonucleotide according to claim 4, characterized in that it codes for a polypeptide with formula (P-K), formula K-(P-K)₄, or with formula $2K(P-K)_4$.
- A recombinant nucleotide sequence comprising a concatenation of nucleotides coding for a plant protein reserve, characterized in that it further comprises an oligonucleotide according to any one of claims 1 to 5, inserted at one site of the nucleotide concatenation selected such that:
 - expression of the nucleotide sequence in a particular plant cell enables a modified protein reserve to be produced which is localised in that cell in

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- the modified protein reserve coded by the recombinant nucleotide sequence is immunologically recognised by antibodies produced against the corresponding normal protein reserve.
- 7. A nucleotide sequence according to claim 6, characterized in that the coding nucleotide concatenation it comprises codes for a protein reserve which is naturally low in lysine.
- A nucleotide sequence according to claim 7, characterized in that the coding nucleotide concatenation it comprises codes for a protein reserve naturally produced by a plant for use in animal or human foodstuffs.
 - 9. A nucleotide sequence according to claim 7 or claim 8, characterized in that the coding nucleotide concatenation it comprises codes for a protein reserve naturally produced by a plant from the cereal family.
 - 10. A nucleotide sequence according to claim 7 or claim 8, characterized in that the coding nucleotide concatenation it comprises codes for a protein reserve naturally produced by a plant from the legume or crucifer family.
- A nucleotide sequence according to claim 9, characterized in that the coding nucleotide concatenation it comprises codes for a maize protein reserve.
 - 12. A nucleotide sequence according to claim 11, characterized in that the coding nucleotide concatenation it comprises codes for a protein reserve from the zein family.
- A nucleotide sequence according to claim 12, characterized in that the coding
 nucleotide concatenation it comprises codes for a protein reserve which is maize γ-zein.
 - 14. A nucleotide sequence according to claim 13, characterized in that the nucleotide concatenation coding for the maize γ-zein it contains has the sequence shown in Figure 9.

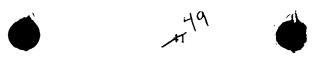
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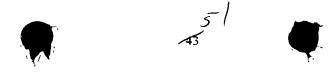
- 26. A lysine-enriched modified maize γ-zein, characterized in that its amino acid sequence is modified by at least one polypeptide with formula (P-K)_n or with formula 2K(P-K)_n, where:
 - n is a whole number of 2 or more,
 - P represents a proline amino acid residue,
 - K represents a lysine amino acid residue;
 - the symbol "-" represents a bond between the two amino acid residues, in particular a peptide type bond, the n (P-K) units being bonded together by bonds, in particular peptide type bonds, said polypeptide having formula (P-K)_n or K-(P-K)_n being substituted for a sequence naturally present in the normal maize γ-zein or being inserted with deletion of one or more amino acids of the amino acid sequence for normal maize γ-zein, or being added to the normal γ-zein amino acid sequence, the insertion site for the polypeptide being selected such that:
 - when the modified lysine-rich γ-zein is produced in a host cell, in particular in a plant cell, it is localised in identical or similar manner to the normal maize γ-zein which would be produced under the same conditions in the same host cell; and/or
 - the modified maize γ-zein is recognised by antibodies directed against the normal maize γ-zein.
- 27. A modified maize γ -zein according to claim 26, characterized in that it is the protein P20 γ Z or the protein H30 γ Z or the protein H45 γ Z.
- 28. A recombinant host cell, characterized in that it comprises a nucleotide sequence according to any one of claims 1 to 17.
- 25 29. A host cell according to claim 28, characterized in that it is a bacterium, for example E. coli or Agrobacterium tumefaciens.
 - 30. A host cell according to claim 28, characterized in that it is a plant cell.
 - 31. A host cell according to claim 30, characterized in that it is a plant seed cell.



- 15. A nucleotide sequence according to claim 7 or claim 8, characterized in that the coding nucleotide concatenation it comprises codes for a protein reserve of a plant selected from the following: soya, sunflower, tobacco, wheat, oats, alfalfa, rice, oilseed rape, sorghum, and <u>Arabidopsis thaliana</u>.
- 5 16. A nucleotide sequence according to claim 13 or claim 14, characterized in that the oligonucleotide is inserted in place of or following the Pro-X domain or in the Pro-X domain naturally present in the maize γ-zein.
 - 17. A recombinant nucleotide sequence, characterized in that it comprises a nucleotide sequence according to any one of claims 1 to 16 under the control of an expression promoter.
 - 18. A recombinant nucleotide sequence according to claim 17, characterized in that the promoter is a specific promoter for a given cell tissue, for example a promoter which is specific for expression in grains, and/or in the leaves of plants.
- 15 19. A nucleotide sequence according to claim 17, characterized in that the expression promoter is that of maize γ-zein.
 - 20. A nucleotide sequence according to claim 17, characterized in that the expression promoter is the promoter CaMV35S.
- A nucleotide sequence according to any one of claims 16 to 20, characterized in that it codes for one of the polypeptides P20γZ or H45γZ with the sequences shown in Figures 11 and 10 respectively.
 - A cloning and/or expression vector, characterized in that it comprises, at a site which is not essential for replication, a nucleotide sequence in accordance with any one of claims 1 to 20.
 - A cloning and/or expression vector, characterized in that it is one of plasmids pP20γZ (CNCM N° I-1640), pH30γZ or pH45γZ (CNCM N° I-1639).
 - A polypeptide coded by a sequence according to any one of claims 1 to 17.
 - A lysine-enriched modified maize γ-zein, characterized in that it is coded by a nucleotide sequence according to claim 13 or claim 14.

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- 32. A host cell according to claim 31, characterized in that it is a cell from maize seed endosperm.
- 33. A host cell according to claim 32, characterized in that it contains a nucleotide sequence according to claim 13, integrated in its genome in a stable manner.
- 34. A host cell according to claim 32, characterized in that it produces a lysineenriched modified maize y-zein according to claim 26 or claim 27.
- 35. A host cell according to claim 30, characterized in that it is a soya, sunflower, tobacco, wheat, oats, alfalfa, rice, oilseed rape, sorghum or Arabidopsis cell.
- 36. Seeds producing a polypeptide according to any one of claims 24 to 27.
- 37. A plant producing a polypeptide according to any one of claims 24 to 27.
- 38. A plant according to claim 37, characterized in that it is a maize plant.
- 39. Seeds obtained from plants according to claim 37 or claim 38.
- 15 40. A method of producing plants or seeds expressing a modified protein reserve, characterized in that it comprises the steps of:
 - a) transforming a plant cell with a nucleotide sequence according to any one of claims 6 to 17, or a vector according to claim 22 or claim 23, under conditions enabling the modified protein reserve coded by the nucleotide sequence to be expressed in a stable and functional manner;
 - b) regenerating plants from the plant cell transformed in step a), to obtain plants expressing the modified protein reserve;
 - c) if necessary, obtaining seeds from the modified plants obtained in stepb).
- A method according to claim 40, characterized in that the plant is maize and the protein reserve is γ-zein.

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